# Effect of water stress on $N_2O$ emission rate of 5 tree species

WANG Miao[1], LI Qiu-rong[2], XIAO Dong-mei[1] WANG Chang-ke[3],
[1] *Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang*, 110016, *P. R. China*
[2] *General Hospital in Nanjing Military District, Nanjing, 210002, P. R. China*
[3] *Institute of Atmospheric Physics*, Chinese Academy of Sciences, *Beijing*, 100029, *P. R. China*

**Abstract:** The $N_2O$ emission rates, photosynthesis, respiration and stomatal conductance of the dominant tree species from broadleaf/Korean pine forest in Changbai Mountain were measured by simulated water stress with the closed bag-gas chromatography. A total of five species seedlings were involved in this study, i.e., *Pinus koraiensis* Sieb. et Zucc, *Fraxinus mandshurica* Rupr, *Juglans mandshurica* Maxim, *Tilia amurensis* Rupr, and *Quercus mongolica* Fisch. ex Turcz.. The results showed that the stomatal conductance, net photosynthetic rate and $N_2O$ emission of leaves were significantly reduced under the water stress. The stoma in the leaves of trees is the main pathway of $N_2O$ emission. $N_2O$ emission in the trees mainly occurred during daytime. $N_2O$ emission rates were different in various tree specie seedlings at the same water status. In the same tree species, $N_2O$ emission rates decreased as the reduction of soil water contents. At different soil water contents (MW, LW) the $N_2O$ emission rates of *Pinus koraiensis* decreased by 34.43% and 100.6% of those in normal water condition, respectively. In broadleaf arbor decreased by 31.93% and 86.35%, respectively. Under different water stresses $N_2O$ emission rates in five tree species such as *Pinus koraiensis*, *Fraxinus mandshurica*, *Juglans mandshurica*, *Tilia amurensis*, and *Quercus mongolica* were 38.22, 14.44, 33.02, 16.48 and 32.33 $ngN_2O \cdot g^{-1}DW \cdot h^{-1}$, respectively.

**Keywords:** Trees; $N_2O$ emission rate; Soil water stress; broadleaf/Korean pine forest; Changbai Mountain
**CLC number:** S718.55 **Document code:** A **Article ID:** 1007-662X(2004)01-0019-05

## Introduction

Global climate change is considered one of the most urgent environmental problems. The main negative impact on climate change is the emission of greenhouse gases ($CO_2$, $CH_4$, and $N_2O$). $N_2O$ emissions not only contribute severely to the greenhouse effect but also lead to the depletion of stratospheric ozone, and it is involving in many photochemical reactions in troposphere. However, some source of $N_2O$ in atmosphere is still unclear. Previously it was considered that biological source of the global atmospheric $N_2O$ was produced during the microbial transformation of nitrate ($NO_3^-$) and ammonia ($NH_4^+$) in soils and water. Recently some reports showed that the plants could release $N_2O$. Chen *et al.* (1990) firstly reported that in normal physiological condition the trees in forest could generate $N_2O$, which was a new source of atmosphere $N_2O$. Therefore, another source of $N_2O$ in terrestrial ecosystems was found. The amount of $N_2O$ emission in forest ecosystem was measured not only from soil flux but also from plants (Xu *et al.* 1995). If the tree itself is involved in $N_2O$ emission and the amount of $N_2O$ emission was determined, then these will provide the new data for the equilibrium of global $N_2O$ sources. Up to date, few studies were found on $N_2O$ emission flux from trees. Some results were available for simulating experiment in the detached leaves and branches of trees (Yang *et al.* 1995). There are great differences in the plants between growing in controlled environment and in natural condition. Therefore, in order to determine the actual $N_2O$ emission of plants, the $N_2O$ emission in the field was measured. But it was seldom reported on $N_2O$ emission of trees in the natural condition (Zhang *et al.* 2002). No report was found in the effects of soil moisture contents on $N_2O$ emission of the tree especially. In our study, by stimulating experiment of 3 levels of soil moisture contents using closed bags, we explored the relationship between $N_2O$ emissions of 5 dominant tree species and their foliar gas exchange during growth seasons. Moreover, our results can provide the data for estimating $N_2O$ emission flux from dominant tree species at high latitudes of Changbai Mountain in light of global change considerations, and predict the change tendency of $N_2O$ emission in forest ecosystem.

## Materials and methods

### Study site and material

The experiment was conducted at the Open Research Station of Changbai Mountain Forest Ecosystem, Chinese Academy of Science (Erdaobaihe Town, Antu County, Changbai Mountain Natural Reserve Zone; 128°28′E, 42°24′N). The altitude of this site is 736 m. Average annual

**Foundation item:** This project was supported by the National Natural Science Foundation of China (No. 30271068), the grant of the Knowledge Innovation Program of Chinese Academy of Sciences (KZ-CX-SW-01-01B-10), and the Special Funds for Major State Basic Research Program of China (No. G1999043407)
**Biography:** WANG Miao (1964-), male, associate professor in Institute of Applied Ecology, Chinese Academy of Science, Shenyang 110016, P. R. China. E-mail: wangmiaojue@163.com
**Received date:** 2003-04-18
**Responsible editor:** Song Funan

temperature is in range of 0.9-3.9 °C. Mean temperature in the coldest month (February) is -16.7-18.6°C and that of in the warmest month (August) is 17.4-20.7°C. This area belongs to a continental mountain climate, affected by monsoon and characterized by a dry and windy spring, warm rainy summer, dry and cold winter. The average annual precipitation is 600-900 mm, with mainly from June to August. Frost-free period is 100-120 d. Detailed information of the study area is provided by Fan *et al.* (1992) and Chi *et al.* (1981). Five tree species were involved in this observation: *Pinus koraienes, Fraxinus mandshurica, Juglans mandshurica, Tilia amurensis,* and *Quercus mongolica.* They were all 6-year-old seedlings.

**Methods**

The 2-year-old seedlings of *Pinus koraienes, Fraxinus mandshurica, Juglans mandshurica* and *Tilia amurensis* were collected from the nursery of Dashiqiao Forestry Bureau in Changbai Mountain in Antu County, Jilin Province. The 3-year-old *Quercuss* seedlings were collected in broadleaf/Korean pine forest of natural reserve zone in Changbai Mountain. On May 15, 2000, the well-grown seedlings with similar association in each tree species were randomly divided into three groups (30 plants per group). And they were planted into three plastic pools that were 100 cm in height, 90 cm in width, and 200 cm in length. All the pools were constructed in the big hob on April 30, 2000. The top of the hob was exposed in sunshine and covered with tarp when it rained. On May 1, 2000 the brown forest soil matrix in each pool was collected from near pine broad leaves forest and the soil matrix depth was 90 cm. On May 25, 2000 the leaves of the seedlings began to develop. According to the natural precipitation and future global precipitation change, we designed three levels of water supply i.e. 600 mm (CK), 450 mm (MW), and 270 mm (LW) during growth season, which corresponded to the precipitation of growth season in temperate broad-leaved forest and temperate grassland as well as grassland, respectively. The seedlings of 5 tree species were cultivated for 3 years. The water treatment began on May 1 and ended on October 1 each year. To avoid the artificial effect on water supply, the seedlings of all treatments were treated with water only in the afternoon of the same day (one times every two days). Thus the total number of watering was 52 times and the amount of water for each times in three water treatments was 11.54 mm, 8.65 mm and 5.19 mm, respectively. During the experiment, all the soil pools were well managed and often weeded.

In situ measurement, the closed bags, made of PVC film with the volume of 2 000 ml, were used in the experiment. From September 4 to October 5, 2002, $N_2O$ emission from the branches and leaves of 5 tree species was measured during 4:00-5:00, 11:00-12:00, and 18:00-19:00 in triplicate. After the leaves and branches were covered with the bags, the gas was sampled from the covered bags by 100 ml plastic injectors in 5 min interval. 10 ml gas was collected each time, sealed and immediately taken to the lab to assay $N_2O$ concentration using HP-5890 gas chromatography. The temperatures of column case and electron capture detector were separately 55°C and 380°C. The carrying gas was highly pure $N_2$ and the rate of flow was 30 $ml \cdot min^{-1}$. $N_2O$ emission rate was calculated by the following formula:

$$F = (M / V_0) \times (V / A) \times (P / P_0) \times (T_0 / T) \times (dC_t / dt)$$

where $F$ is $N_2O$ emission rate of the leaves inside the covered bags; $M$ is the molar mass of $N_2O$, $V_0$ is the molar volume of the gas under standard condition; $P$ and $P_0$ are the air pressures in sampling site and in standard condition, respectively; $T$ and $T_0$ are separately the air temperature in sampling and absolute temperature in air under standard condition; $dC_t$ is the mixed specific concentration of $N_2O$ volume, and $t$ is time.

After sampling, the characteristics of gas exchange in the leaves of the same branches were determined by using the portable gas exchange system (LI-6400 portable photosynthesis system, Li-Cor Inc., Lincoln, NE, USA), with a gas rate of 500 $ml \cdot min^{-1}$. The photosynthetic parameters such as leaf net photosynthetic rate ($p_n$), respiration rate (R), evaporation rate (E), stomatal conductance (gs), photosynthetic efficiency radiation (PAR), and intercellular $CO_2$ concentration ($C_i$) were also measured. The photosynthesis curve responding to light (PPFD) in the leaves of seedling was measured using LED red and blue light (6400-02B leaf chamber). At early stage of the measurement, the light intensity was very high, and then reduced gradually. Under the same light intensity, $p_n$ was measured in triplicate. Afterwards, the branches tested were cut down. The volume of covered bags (including the branches and leaves of the trees) and the volumes of the branches and leaves were measured by water depletion method. The available capacity of gas in the covered bag was different between both of the volumes. The leaf area inside the bags was measured using LI-3000 leaf area meter (LI-COR Biosciences, U.S.A). The samples were dried at 105°C for 30 min to a constant weight at 80°C, and weighed with AP250D electric balance (Ohaus Company, Switzerland).

**Statistical analysis**

Variance analysis and Duncan multiple-range test were performed by the SigmaStat 2.03 procedure of SAS (SAS, 1995). One-way ANOVA analysis of variance was preformed for the results including growing and physiological parameters, and the significance level was tested.

## Results and discussion

**The change of $N_2O$ emission rate of the trees**

On September 4, 2002 $N_2O$ emissions from the seedlings of 5 dominant tree species in broadleaf/Korean pine forest in Changbai Mountain were measured in situ. Since $N_2O$

emission of the trees was mainly influenced by the environmental factors and its daily change did not have certain regularity, therefore, the mean values of $N_2O$ emission of the 5 tree species in 3-time intervals during the daytime were used to observe the change of $N_2O$ emission rate under soil water stress. As shown in Fig.1 (a, b), in the same level of soil water content $N_2O$ emission rate of 5 tree species was obviously different. Averagely, $N_2O$ emission rate of the 5 tree species in CK group varied from 14.45 to 38.22 $ngN_2O\cdot g^{-1}$ $DW\cdot h^{-1}$, which was about 32.72% and 86.26% higher than those in MW (4.23-27.62 $ngN_2O\cdot g^{-1}$ $DW\cdot h^{-1}$) and LW groups (-1.35-8.58 $ngN_2O\cdot g^{-1}$ $DW\cdot h^{-1}$), respectively. In CK group, $N_2O$ emission rate of the 5 tree species followed the order of *Pinus koraiensis*> *Juglans mandshurica*> *Quercus mongolica*> *Tilia amurensis*> *Fraxinus mandshurica.* The results were consistent with the studies of Zhang (2002) for *Pinus koraiensis* and *Fraxinus mandshurica.* It was mainly due to a decline of the temperature in Changbai Mountain since September. The tree growth was not as active as in summer. But for *Pinus koraiensis*, one of the evergreen plants, its metabolic activities are not sensitive to temperature. Our results were also relative to the characteristic of N fixation in root system. The $N_2O$ emission rate of *Pinus koraiensis* seedlings was the highest, but there were no significant differences between *Pinus koraiensis* and *Juglans mandshurica* and *Tilia amurensis.* In MW group, $N_2O$ emission rate of the different trees species followed the order of *Quercus mongolica*>*Juglans mandshurica*>*Pinus koraiensis*>*Fraxinus mandshurica*> *Tilia amurensis.* There were no significant differences between *Quercus mongolica* and *Juglans mandshurica* and *Pinus koraiensis* ($P$<0.05), and that there were significant differences between *Fraxinus mandshurica* and *Tilia amurensis* ($P$<0.05). In LW group, the results were different from those in MW and CK groups. $N_2O$ emission rates of *Juglans mandshurica*, *Quercus mongolica*, and *Tilia amurensis* were remarkably higher than those of *Pinus koraiensis* and *Fraxinus mandshurica.* During the whole experiment of CK group, the mean values of $N_2O$ emission rate of the 5 tree species were still high in September, which were close to the level of those in the soil of broadleaf/Korean pine forest (39 $ngN_2O\cdot g^{-1}$ $DW\cdot h^{-1}$) (Xu *et al.* 1995). Therefore, the source of $N_2O$ emission from the 5 tree species cannot be ignored in the ecosystem of broadleaf/Korean pine forest. And the 5 tree species distributed widely in the northern China, thus we should pay more attention to them. As the development of drought tendency of climate, $N_2O$ emission of trees decreased gradually. Moreover, $N_2O$ emission of different tree species took on a decreasing tendency with the decline of soil water content (Zhang 2002). The responses of different species to $N_2O$ emission and soil water stress were different, which maybe were affected by multiple factors such as the physiological characteristics of plants and environmental conditions. Our results demonstrated that for photophilic tree species including *Pinus koraiensis*, *Quercus mongolica* and *Juglans mandshurica*, $N_2O$ emission was relatively higher than those of shade species. Because the respiration of photophilic plants is stronger than those of the shade species, moreover, the photophilic species are able to offer more energy in the process of nitrate assimilation and dissimilation for themselves than the shade species do. The present study showed that the trees could release $N_2O$ and the amount of $N_2O$ emission reached such a high level just as in soil (Zhang 2002). So it is incorrect in the evaluation of $N_2O$ emission of forest ecosystem without considering the source of $N_2O$ emission from the trees. It is also considered as one of the reasons leading to imbalance of source and sink of $N_2O$ emission.

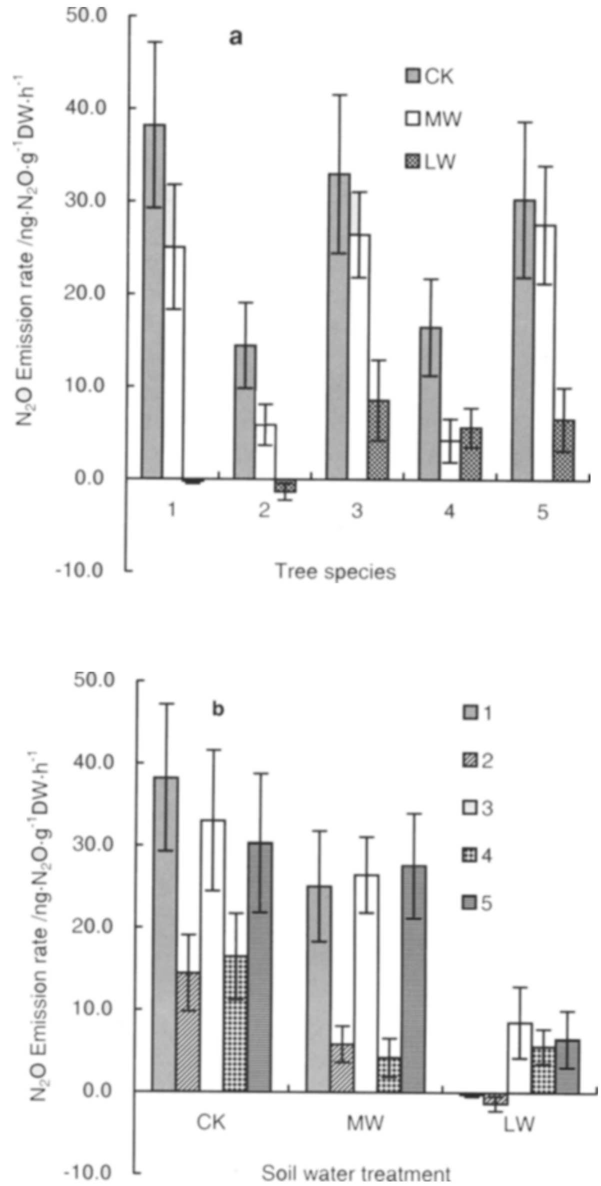


**Fig.1 Effect of water stress on $N_2O$ emission rate of 5 tree species**

1 *Pinus koraiensis*, 2 *Fraxinus mandshurica*, 3 *Juglans mandshurica*, 4 *Tilia amurensis*, 5 *Quercus mongolica*

### Leaf $CO_2$ exchange, stomatal conductance and $N_2O$ emission under soil water stress

*Foliar stomatal conductance and $N_2O$ emission*

The gas exchange of water vapor and $CO_2$ between plants and atmosphere is mostly carried out through the stomata in leaves. The stomata opening which affect the rate of gas exchange is regulated by many factors. The better understanding for $N_2O$ emission to pass through the stomata or not is useful to explain the relationship between $N_2O$ emission and other physiological activities. As shown in Table 1, the stomatal conductance and $N_2O$ emission rate of 5 tree species were greatly affected by soil drought treatments, however their change tendencies were identical among the different soil water stress. At the same time in the diurnal variation experiment, though the change of $N_2O$ emission rate of the trees was bigger and in some cases $N_2O$ might be absorbed by the trees, which indicated the stomata opening in the leaves was related to $N_2O$ emission flux, and the stomata is the main pathway of $N_2O$ emission from trees.

*Leaf $CO_2$ exchange and $N_2O$ emission*

The photosynthesis, stomatal conductance, and $N_2O$ emission rate of 5 tree species were measured under different soil water stress. The results showed that the net photosynthetic rate of the 5 tree species was clearly inhibited by soil water stress (Table 1). As the reduction of soil water content, the net photosynthetic rate also had certain extents of decreases. And the result was further analyzed by Duncan multiple-range test. The net photosynthetic rate was significantly different between 4 tree species, i.e, *Pinus koraiensis*, *Juglans mandshurica*, *Tilia amurensis*, and *Quercus mongolica*, among different soil water treatments ($P<0.05$). While for *Fraxinus mandshurica*, there was no significant difference between CK and MW groups ($P<0.05$) and the net photosynthetic rate decreased remarkably as the reduction of soil water content. The results indicated that the photosynthesis was significantly affected by soil water, and $N_2O$ emission of the trees was greatly inhibited by soil water stress. Soil water can enhance $NO_3^-$ transport from soil to the leaves of plants (Xu *et al.* 1990). Chen *et al* (1999) found that $N_2O$ emission rate of *Lolium perenne* L. was positively correlated to the level of $NO_3^-$ in the leaves, which showed that $NO_3^-$ induced the activity of nitric acid reductase (NR) in the leaves (Xu *et al.* 1990). The enzymes involving in $NO_3^-$ assimilation include nitric acid reductase (NR) and nitrous acid reductase (NiR) etc.. NR firstly catalyzes the reduction of $NO_3^-$, which is the key step in assimilation. The NR activity is regulated by many factors and inhibited under soil water stress (Sinha and Nicholas 1980). Moreover, the energy decrease of nitrate assimilation generated by photosynthesis leads to the decline of NADH activity. And then the proteins synthesis is inhibited, which affects $N_2O$ emission from the dissimilation in plants. The microorganism process of soil $N_2O$ production was affected by the soil water stress. Wang *et al* (2001) reported that $N_2O$ emission flux in soil was correlated to soil water content in the plain. Such result was also observed by soil culture from broadleaf/Korean pine forest in Changbai Mountain (Zhang *et al.* 2001). A few studies demonstrated that $N_2O$ in soil could be transported to the atmosphere by the plants (Chang *et al.* 1998; Rusch and Renneberg 1998). Soil water stress not only reduced soil $N_2O$ transport but also decreased $N_2O$ emission of plants themselves. The both effects also reduced the $N_2O$ emission from the branches and the leaves of the trees.

**Table 1. Effect of soil water stress on photosynthetic rate, respiration rate, transpiration rate, stomotal conductance and $N_2O$ emission of 5 tree species**

| Species | Treatments | Net photosynthetic rate /$\mu mol \cdot m^{-2} \cdot s^{-1}$ | Stomatal conductance /$\mu mol \cdot m^{-2} \cdot s^{-1}$ | $N_2O$ emission rate /$ngN_2O \cdot g^{-1}DW \cdot h^{-1}$ | Respiration rate /$\mu mol \cdot m^{-2} \cdot s^{-1}$ |
|---|---|---|---|---|---|
| | CK | 10.654±1.557b | 0.142±0.016b | 42.218±6.634 | -1.097±0.213c |
| *Pinus koraiensis* | MW | 12.480±1.650a | 0.184±0.016a | 28.218±1.342 | -1.328±0.356ab |
| | LW | 7.887±1.008c | 0.108±0.008c | 2.254±0.9731 | -1.578±0.153a |
| | CK | 6.078±0.347a | 0.088±0.010a | 15.463±3.473 | -0.875±0.049c |
| *Fraxinus mandshurica* | MW | 6.548±0.463a | 0.064±0.011b | 5.874±3.122 | -1.145±0.121b |
| | LW | 5.643±0.964b | 0.0181±0.005c | -5.647±2.121 | -1.537±0.323a |
| | CK | 12.383±0.713a | 0.302±0.016a | 33.021±7.12 | -1.108±0.394c |
| *Juglans mandshurica* | MW | 5.497±0.365b | 0.042±0.006c | 18.582±3.323 | -1.891±0.402ab |
| | LW | 4.077±0.865c | 0.060±0.016b | 26.465±4.542 | -2.178±0.406a |
| | CK | 8.868±0.153a | 0.143±0.009a | 16.489±4.322 | -0.916±0.164c |
| *Tilia amurensis* | MW | 8.288±0.273b | 0.095±0.002b | 5.849±2.123 | -1.110±0.074b |
| | LW | 5.989±0.361c | 0.072±0.002c | 5.639±3.211 | -1.574±0.130a |
| | CK | 4.727±1.032a | 0.077±0.007b | 32.330±5.221 | -1.444±0.724b |
| *Quercus mongolica* | MW | 3.263±0.648b | 0.104±0.001a | 29.624±4.231 | -2.016±0.491a |
| | LW | 2.433±0.422c | 0.057±0.003c | 8.573±1.348 | -1.411±0.341bc |

**Note**: Data of the Table are average value. Treatment with the same letters is not significantly different ($P<0.05$) according to Duncan multiple range test.

## Conclusion

$N_2O$ emission was observed in ligneous plants such as conifer and broadleaf tree species and it was varied from tree species to tree species. Averagely, $N_2O$ emission rates of *Pinus koraiensis*, *Fraxinus mandshurica*, *Juglans mandshurica*, *Tilia amurensis* and *Quercus mongolica* were 38.22, 14.44, 33.02, 16.48, and 32.33 $ngN_2O \cdot g^{-1}DW \cdot h^{-1}$, respectively. Of them, the tree species of the maximum $N_2O$ emission rate was *Pinus koraiensis*, and that of the minimum was *Tilia amurensis* and *Fraxinus mandshurica*.

$N_2O$ emission was significantly inhibited by soil water stress. In MW and LW groups, $N_2O$ emission rate decreased by 47.74% and 85.52% comparing with CK group. The balance of source and sink of $N_2O$ emission in forest ecosystem can be changed by soil drought stress.

$N_2O$ emission rates of conifer and sclerophyllous trees were higher than those of deciduous trees, which resulted from the difference of physiology and metabolism among the tree species. The stomata are the main pathway of $N_2O$ emission in the trees.

## References

Chang, C., Janzen, H.H., Cho, C.M. & Nakonechny, E.M. 1998. Nitrous oxide emission through plants [J]. Soil Science Society of America Journal, **62**: 35-38.

Chen Guanxong, Shang Chuhui, Yu Kewei, *et al.* 1990. Investigation on the emission of $N_2O$ by plants. Chinese Journal of Applied Ecology [J], **1**: 94-96. (in Chinese)

Chen, X., Boeckx, P., Shen, S.M., Van Cleemput, O.V. 1999. Emission of $N_2O$ from rye grass (*Lolium perenne* L.) [J]. Biology and Fertility of Soils, **28**: 393-396.

Chi Zhenwen, Zhang Fengshan, Li Xiaoyan. 1981. The primary study on water-heat conditions of forest ecosystem on northern slope of Changbai Mountain [J]. Research of Forest Ecosystem, **II**: 167-178. (in Chinese)

Rusch, F. & Renneberg, H. 1998. Black alder (*Alnus glutinosa* (L.) Gaertn.) tress mediate methane and nitrous oxide emission from the soil to the atmosphere [J]. Plant and Soil, **201**: 1-7.

Sinha, S.K. & Nicholas, D.J.D. 1981. Nitrate reductase [M]. Paleg and Aspinall, p145-171.

Wang Yuesi, Ji Baoming, Huang Yao. 2001. Effect of grazing and cultivating on emission of nitrous oxide, carbon dioxide and uptake of methane from grasslands [J]. Environmental Science, **22**(6): 7-13.

Xu Hui, Zhang Xiujun, Ma Chengxin. 1995. A preliminary study on $N_2O$ and $CH_4$ emission from different soils on northern slope of Changbai Mountain [J]. Chinese Journal of Applied Ecology, **6**: 373-377. (in Chinese)

Xu Changai, Ni Jishan. 1990. The regulations of upper transportation of $NO_3^-$ and outflow of $NO_3^-$ in a vacuole on nitrate reductase activity [J]. Acta Phytophysiologica Sinca, **16**: 337-340. (in Chinese)

Yang Sihe, Chen Guanxong, Lin Jihui, *et al.* 1995. $N_2O$ emission from woody plants and its relation to their physiological activities [J]. Chinese Journal of Applied Ecology, **6**: 337-340. (in Chinese)

Zhang Xiujun, Xu Hui, Chen Guanxong. 2001. Effect of soil moisture and temperature on $CH_4$ and $N_2O$ emission of forest soil [J]. Journal of Forestry Research, **11**(3): 203-206.

Zhang Xiujun, Xu Hui, Chen Guanxong. 2002. $N_2O$ emission rate from trees [J]. Acta Phytoecilogica Sinica, **62** (5): 538-542.